Review

Natural product chemistry and its part in the defence against insects and fungi in agriculture†

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Abstract: This paper surveys our work on natural products as potential models for defensive substances against insect and fungal predators. Insecticides and repellents included are pyrethrins, rotenoids, lipid amides, phorbol esters, cordifolia germacranolides, nicandrenoids, mammeins, dihydroagarofuran esters, and cembrene diols. The fungal H-S toxins from *Alternaria*, and avenacins from oat roots are briefly considered. The avenacins provide an in-situ defence of oat roots against the destructive 'Take-all' fungus disease.

Keywords: natural insecticides; insect repellents; fungicides

1 INTRODUCTION

An organic chemist in academia has a wide choice of problems, assuming that the necessary finance is available, but personally I have always had an interest in some of the problems of agriculture. This has been stimulated by a long-standing friendship with Dr Michael Elliott, formerly of Rothamsted Experimental Station, a period in Dr John Casida's laboratory in Berkeley which stimulated new ways of considering the problems of insecticides, and periods of consultancy to Shell and Wellcome in the days when they were active in agrochemicals. I particularly valued my contacts with Dr Malcolm Black and his Wellcome group.

2 THE NATURAL PYRETHRINS

My first research acquaintance with insecticidal natural products was when I joined Dr Stanley Harper and his two research students Michael Elliott and Hugh Reed at Kings College, London. We were PhD students researching on the natural pyrethrins from the daisy-like flower heads of *Chrysanthemum cinerariaefolium* Vis, now known to contain six important insecticidal components. Three of these are pyrethrin I (Fig 1; **1**), cinerin I (**2**) and jasmolin I (**3**), whilst the

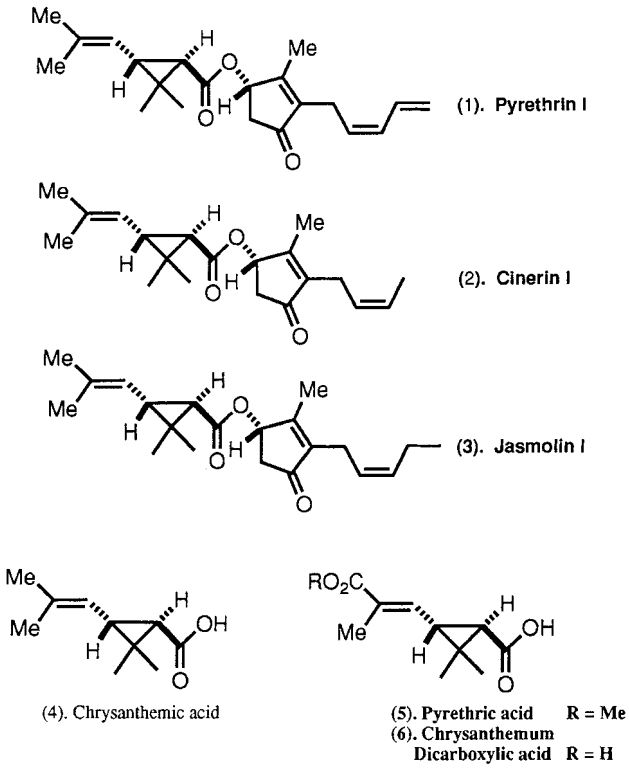

Figure 1. The natural pyrethrins and cyclopropane fragments.

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† Based on a lecture given by the author at an American Chemical Society Symposium 'Recent Progress in Fundamental Research for the Chemical Control of Insects' organised by Dr John Casida and Dr Michael Elliott and held in Boston, USA, in August 1998, at which the author was presented with the ACS International Award for Research in Agrochemicals
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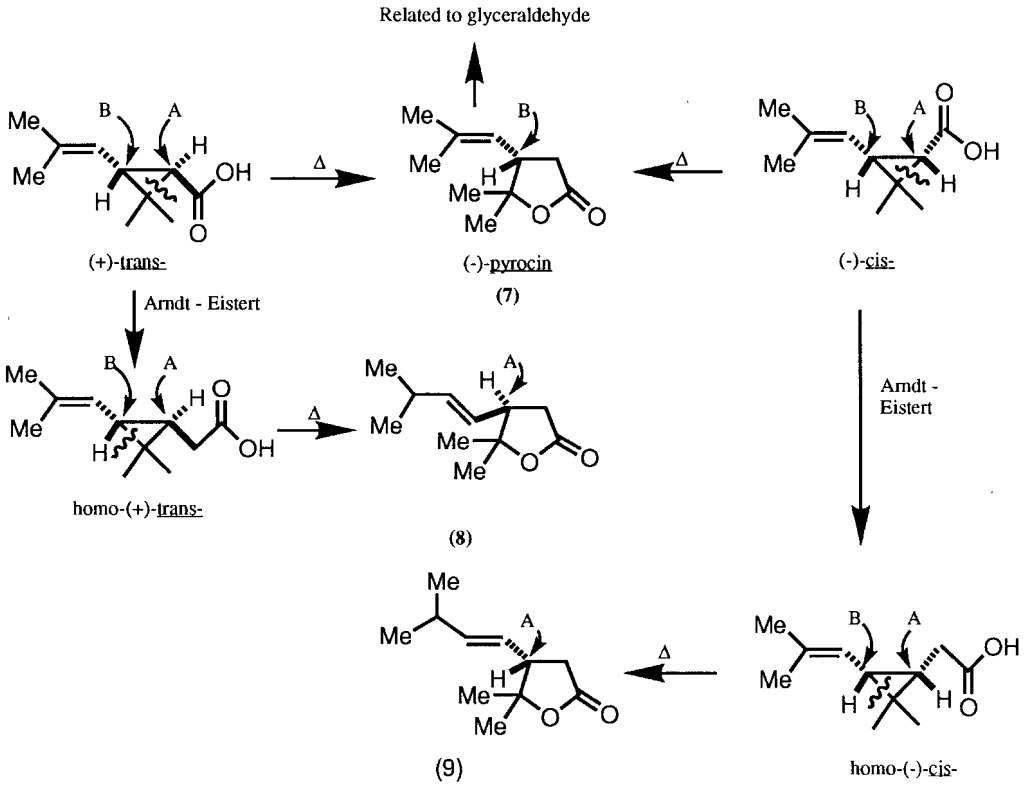


Figure 2. Absolute configurational relationships of the chrysanthemic acids.

other three belong to the II-rethrin series in which the (+)-*trans*-chrysanthemic acid (**4**) portion of the ester is replaced by pyrethric acid (**5**), the side-chain methyl ester of chrysanthemum dicarboxylic acid (**6**).[1] At this stage Dr (later Professor) S H Harper, in collaboration with Dr Ishbel Campbell of Southampton University, had separated and optically resolved synthetic *cis*- and *trans*-chrysanthemic acids. Our later studies on the pyrocins formed on pyrolysis of the acids enabled absolute configurations to be determined; we also cleared up some stereochemical points relating to the geometry and nature of the side chain of pyrethric acid, and of the pyrethrin ketol side chains.

The way Harper and I determined for the first time the absolute configuration of the chrysanthemic acids is shown in Fig 2.[2,3] (+)-*trans*- and (−)-*cis*-Chrysanthemic acids both give on pyrolysis the same (−)-pyrocin (**7**), the absolute configuration of which can be linked to a glyceraldehyde standard. Centre A is destroyed and B retained so both stereoisomeric chrysanthemic acids have the same configuration at centre B. On the other hand, if a methylene is inserted between the carboxyl and the cyclopropane using the Arndt–Eistert homologation reaction as shown in Fig 2, pyrolysis gives two enantiomeric lactones (**8**) and (**9**), destroying centre B and retaining A. They thus have opposite configurations at A.

The first completely synthetic pyrethrins to be made were tetrahydropyrethrin-I and dihydrocinerin I, prepared in our laboratory as diastereoisomeric mixtures (by reaction between the appropriate (±)-bromocyclopentenone and the silver salt of (±)-*trans*-chrysanthemic acid, a method which did not accommodate unsaturated ketol side chains).[4,5] Later, we made pyrethrin I,[6,7] cinerin I[8–10] and jasmolin I[10] in *cis*-(*Z*)- and *trans*-(*E*)- forms as diastereoisomeric pairs using a pyruvaldehyde route[11] and esterifying with (±)-*trans*-chrysanthemic acid. A rethrin II specimen was also made using pyrethric acid.[12] Jasmolin I at the time of its synthesis was not known to be part of the active pyrethrins mixture and its presence was discovered by my former research student Peter Godin.[13] All the stereochemical points including absolute configuration were eventually verified by an X-ray structure (Fig 3) of the synthetic rethrin derivative (**10**)[14] which linked all the natural rethrins *via* their CD (and ORD) curves (Fig 4).[15] During the synthetic work we made the perfume component jasmone (see Fig 8) in (*Z*)- and (*E*)- forms (from the point of view of odour, the natural (*Z*)- form is much superior to the (*E*)-)[16] and later we studied further aspects of the chemistry and spectra of the pyrethrins.[17–22] During this period Michael Elliott went to Rothamsted to begin his pioneering pyrethroid work, using the natural pyrethrins as his initial model, and Hugh Reed left for a distinguished career in ICI. I took up a lectureship at Imperial College, London, collaborating with Harper at Kings College London in order to complete our work.

3 BIOSYNTHESIS AND THE PYRETHRINS

My group has always been interested in the biosynthesis of natural products and our synthesis of the

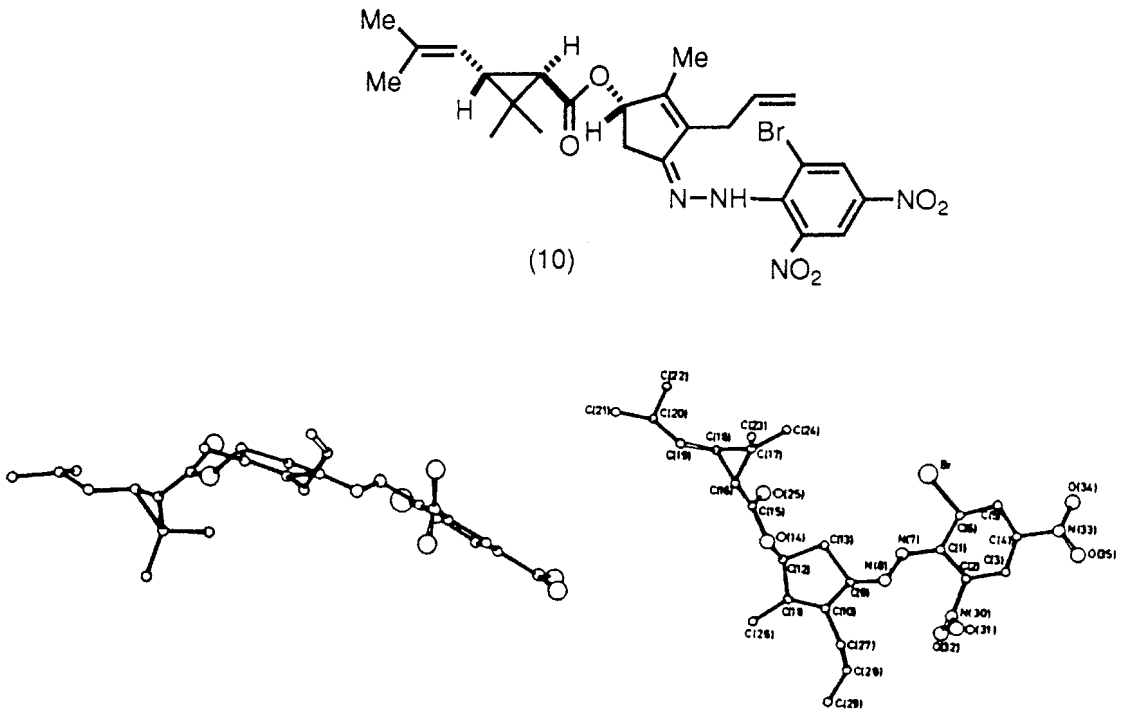


Figure 3. X-ray structure of (+)-allylrethronyl-(+)-*trans*-chrysanthemate -6-bromo-2,4-dinitrophenylhydrazone (two views).

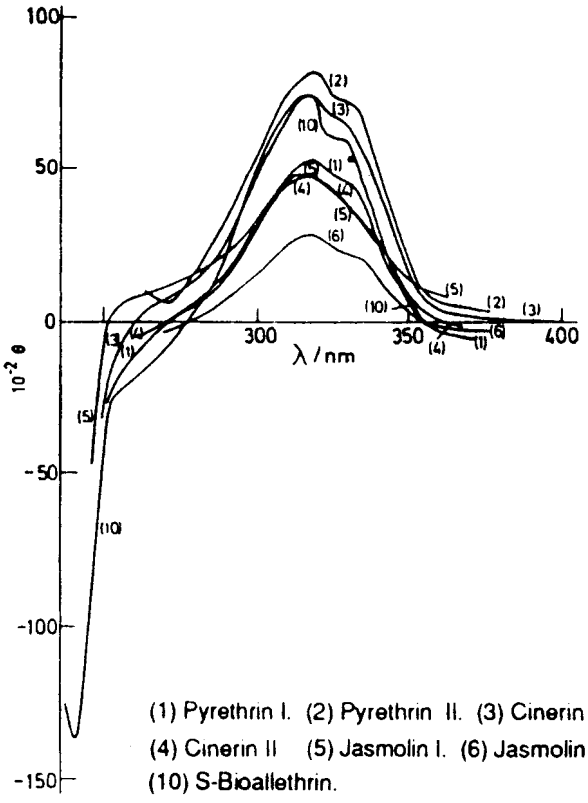


Figure 4. Circular dichroism spectra for pyrethrins.

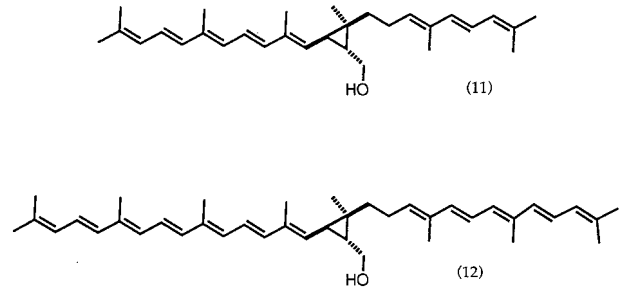


Figure 5. Presqualene (11) and prephytoene (12).

cyclopropanes presqualene (Fig 5; **11**)[23] and prephytoene (**12**),[24] immediate precursors to squalene and phytoene, arose from these early interests in chrysanthemic acid. Plausible biosynthetic schemes for chrysanthemic acid and presqualene have been proposed.[1]

Much less is certain about the origins of the ketols although I have always believed they were possibly fatty-acid derived. However, feeding fatty acids to a crude enzyme preparation from the achenes of pyrethrum flowers did not produce the ketols, as the fatty acids were degraded and reincorporated mainly as acetate.[25]

Later, we were engaged on an isotopic study of the enzymic oxidation reactions of linolenic acid (**13**) in which the C–C chain is fractured,[26,27] and became interested in the demonstration by Vick and Zimmerman that linolenic acid hydroperoxide (**14**) was also the precursor of a new compound 12-oxophytodienoic acid (12-oxo-PDA) (**16**) formed via an allene epoxide (**15**) (Fig 6).[28–30] 12-oxoPDA, in its turn, was the precursor of *cis*- or epi-jasmonic acid (Fig 7; **17**) (which is easily isomerised to the *trans*- isomer (**18**)). Enzymic reduction and three β-oxidations are involved. We have synthesised all the compounds of the 12-oxo-PDA cascade,[31] and it has become realised in recent years that jasmonic acid is an important and

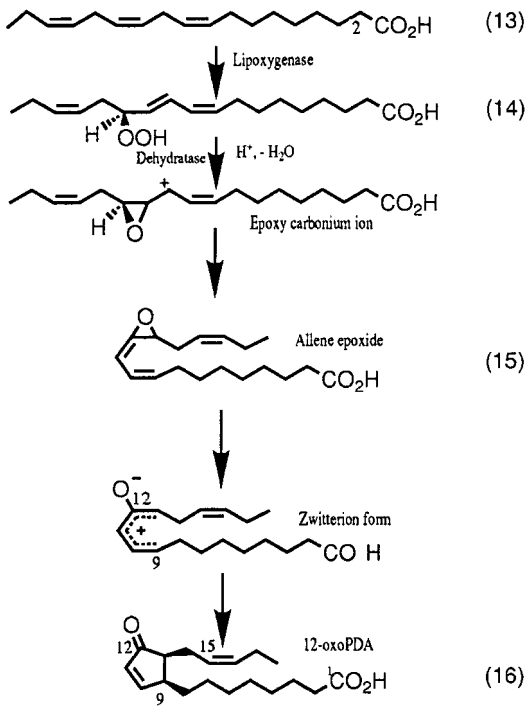


Figure 6. Biosynthesis of 12-oxoPDA from linolenic acid.

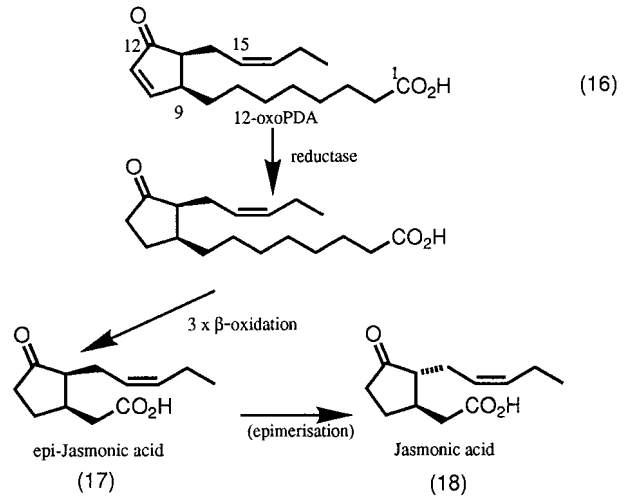


Figure 7. Reduction and β-oxidation of 12-oxoPDA leading to epi-jasmonic acid and jasmonic acid.

widely distributed hormone of plants and fungi, being concerned with growth-regulating and senescence-inducing properties. It is also involved in wound healing, growth inhibition, seed germination inhibition, potato tuber induction etc. Following formation of jasmonic acid and its action as a hormone, the hormone has to be deactivated and eliminated, and we have suggested that jasmonic acid is the precursor of jasmone through dehydrogenation, double bond migration and decarboxylation as a vinylogous β-keto acid (Fig 8).[1] (*Z*)-Jasmone (**19**) is a component of many flower perfumes and its volatility may be an excretory mechanism.[32] By the same token, pyrethrin ketols might be viewed as products of metabolic 4-hydroxylation of jasmone (**19**) to jasmolone (**20**), along with side-chain modification (processes for which there is microbiological analogy in the literature).[1] It may therefore be that pyrethrins are excretory products destined to fall from the plant in the achenes as the flower dies. More work in this area is needed to see if such ideas are well-founded.

4 THE DUCKWEED ALGICIDE

Before leaving the 12-oxoPDA story I thought it might be appropriate to mention that we have synthesised the

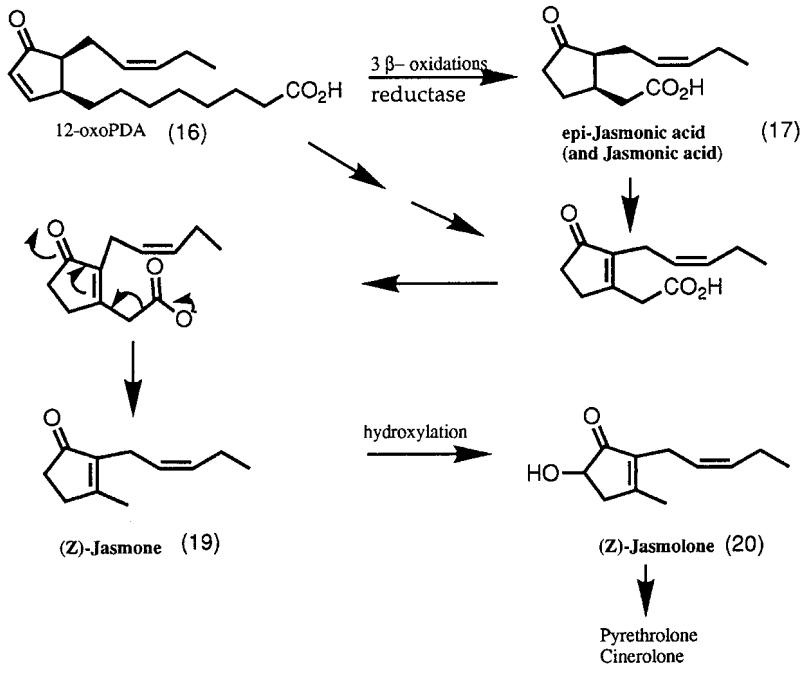


Figure 8. Proposal for the formation of epi-jasmonic acid, jasmonic acid, jasmone, jasmolone and other pyrethrin ketols.

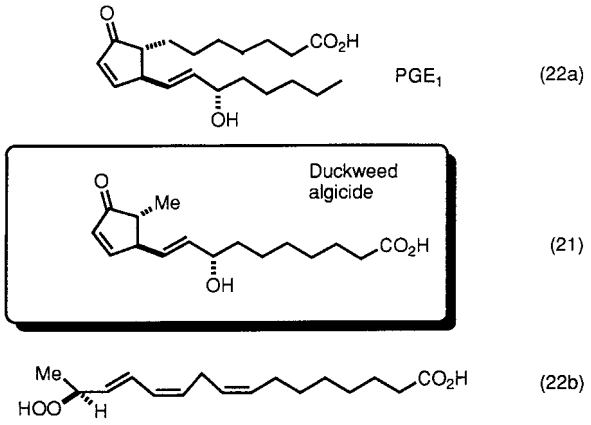


Figure 9. Duckweed algicide and possible relatives.

molecule (**21**) (Fig 9).[33] It is produced by duckweed, *Lemna trisulca* L as an algicide and its function is to keep the photosynthetic surface clear of screening by algae.[34] The cyclopentenone (**21**) does have a structural resemblance to an animal prostaglandin eg PG E_1 (**22a**) (which belongs to the *trans*-cyclopentene series), but an alternative more consistent with the plant 12 oxoPDA route (cf Fig 6), involves the hydroperoxide (**22b**) as precursor, along with methyl epimerisation. Further studies are required to elucidate the biosynthesis.

5 THE ROTENOIDS

The rotenoids, the active components of the insecticide derris, are a second long-standing interest in our laboratory The best known rotenoid rotenone (**23**), inhibits the respiratory electron transport chain and although not a large-volume insecticide it is also valuable as a fish poison for restocking waters with more desirable species. Rotenoids we use in our biosynthetic work are shown in Fig 10 (**23**)–(**26**). The

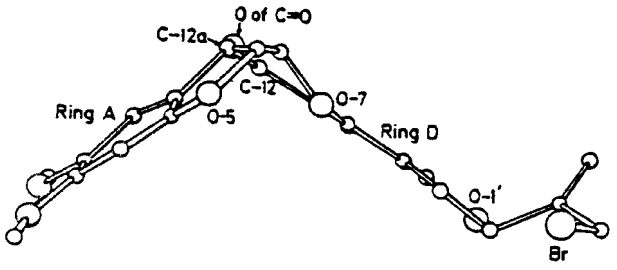


Figure 11. X-ray structure of 8′-bromo-(6a*S*, 12a*S*, 5′*R*)-rotenone.

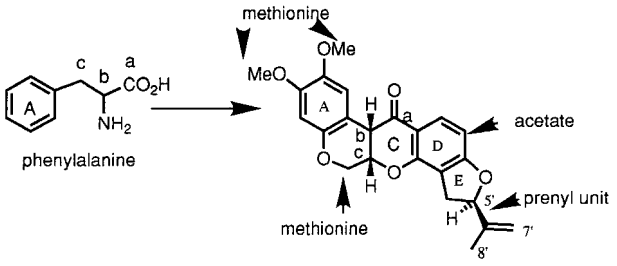


Figure 12. Precursors for the gross biosynthetic make-up of rotenone.

molecules are bent, as the X-ray structure (Fig 11)[35] demonstrates, and our early work was concerned with chemistry and stereochemistry.[36] More recently rotenoid biosynthesis has been a major interest in our laboratory. In studying the biosynthesis of the four rotenoids (**23–26**) we have used the three plants *Derris elliptica* Benth, *Amorpha fruticosa* L and *Tephrosia vogelii* Pers. Compared with the pyrethrins, much less is known about structure–insecticidal activity relations in this group, but a good deal is now known about the biosynthetic pathway–an investigation that has occupied us for many years. In very gross terms the metabolic make-up for rotenone is as shown in Fig 12,[37] but we have studied the processes in some detail,

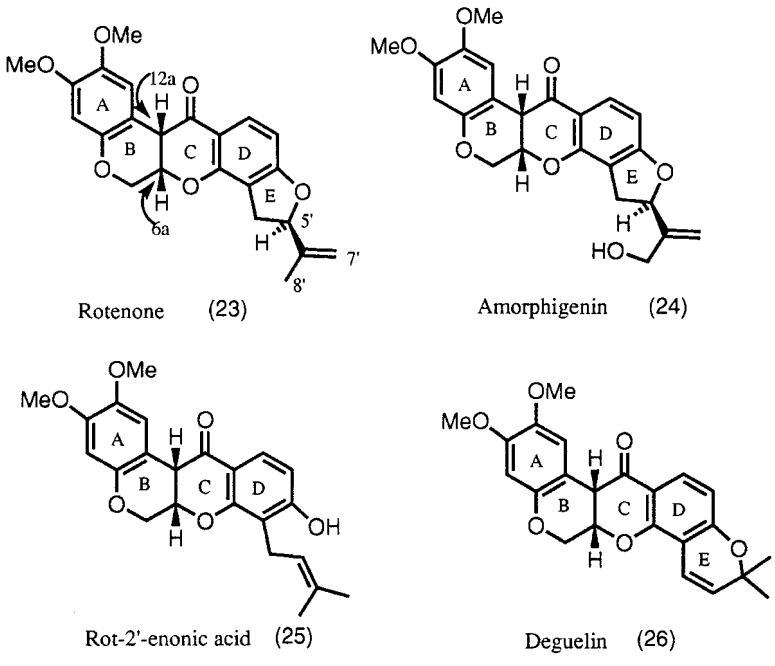


Figure 10. Rotenoids employed in the biosynthetic investigation.

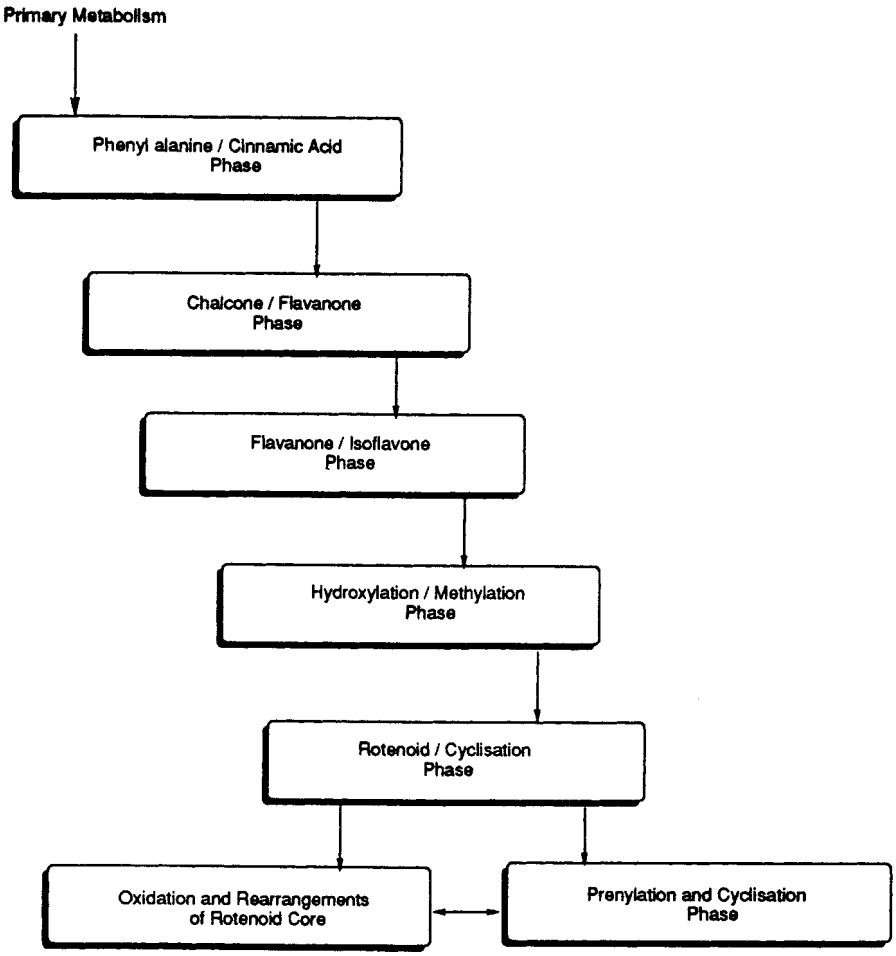


Figure 13. Phases in the biosynthesis of rotenoids.

taking the biosynthesis through a series of metabolic phases, using, in particular, isotopic labelling methods. Each box of Fig 13 contains the chemistry relevant to the particular enzymic changes. However I shall not go into further detail as a full account of our work has recently appeared in *Phytochemistry*.[38] Instead I wish to refer briefly to other natural products we have worked on, which may provide models for usable synthetic insecticides in the future.

6 UNSATURATED AMIDE INSECTICIDES

One such promising group is the isobutylamides and related structures. These were tested for us initially by Rothamsted, although we had first to correct a number of defective early chemical structures. Neoherculin (Fig 14; **27**) is a typical example of a natural insecticide in this class, isolated from *Zanthoxylum clava-herculis* L, carrying a *trans*-(*E*)-α-unsaturated isobutylamide function separated by two methylenes from a *cis*-(*Z*)-ene or -polyene grouping.[39] Anacyclin (**28**) from *Anacyclus pyrethrum* (L) Link only becomes insecticidal when the two acetylenes are stereospecifically reduced to a (*Z*),(*Z*)- diene.[40] In later work we have concentrated on synthetic work for structures and synthons which we know carry the elements of insecticidal activity,[41–43] and hydrozirconation methods have been very useful here.[44,45]

Unfortunately the stability of the natural aliphatic

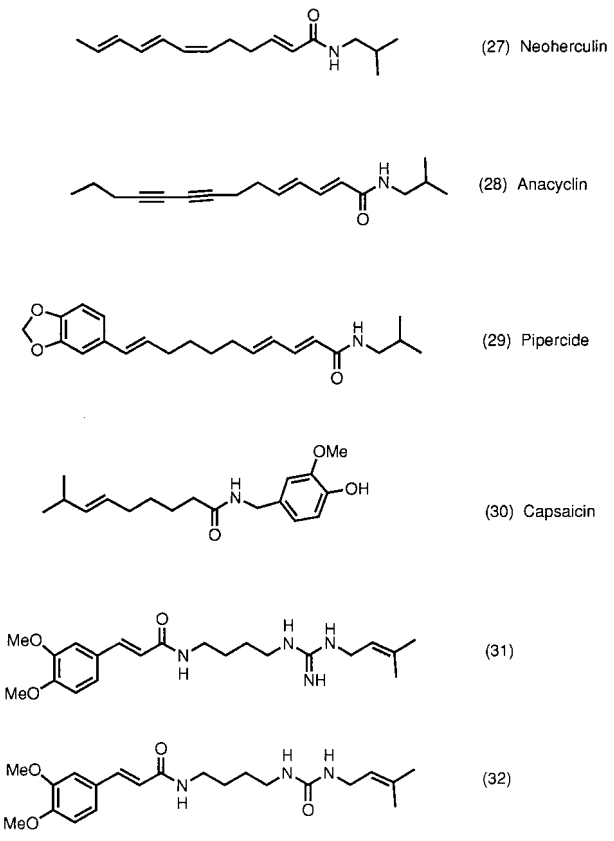
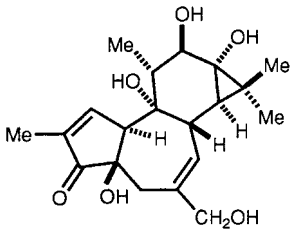
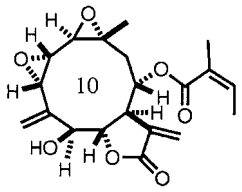


Figure 14. Compounds associated with work on unsaturated amide insecticides.

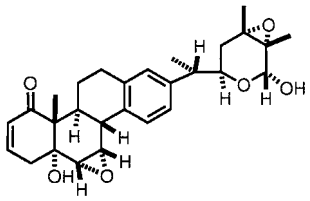


(33) Phorbol

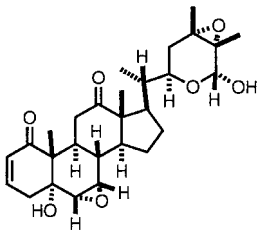


(34) Cordifine

Figure 15. Structures of phorbol and cordifine.



(35) Nic-1



(36) Nic-7

Figure 16. Two representative Nicandra steroids.

compounds is poor, but Miyakado has shown that aromatic substitution can greatly improve this – as in pipericide (**29**) from black pepper (*Piper nigrum* L).[46] The vanillylamide capsaicin (**30**), the active principle of hot red peppers, which we synthesised many years ago,[47] is not an insecticide. The guanidine (**31**), a natural hypotensive from *Verbesina caracasana* L, and the amide (**32**) have been synthesised in our laboratory in (Z)- and (E)- forms,[48] but these have not yet been investigated as possible insecticides. Dr Robert Blade, formerly of Wellcome, reviewed the area a few years ago[49] and there are one or two recent patents in the literature. It would not surprise me if a commercially significant insecticide emerges one day from these models.

7 CROTON OIL

Phorbol esters represent the active principles of the oil from *Croton tiglium* L seeds and our concern has been structure and chemistry of the diterpene phorbol (Fig 15; **33**) itself.[50] The enzyme protein kinase C, important in cellular signal transduction, and apparently concerned in cell growth patterns which may lead to tumour promotion, is strongly activated by phorbol 12-myristate-13-acetate.[51]

There have been various early reports of the insecticidal action of croton oil[50] and it would be interesting to know more of the way this vesicant oil and its phorbol esters act as insecticides.

8 THE CORDIFINE GROUP

Extractives of *Erlangea cordifolia* Moore, a Kenyan member of the Compositae, are reported to be effective anti-feedants towards the army worm, and our investigation showed that the plant produced six germacranolides. The structure and absolute configuration of one of these, cordifine (Fig 15; **34**) (as its 5-bromo-2-furoate ester), was determined by single crystal X-ray crystallography; the remainder of the closely related five structures followed from NMR spectroscopy and further X-ray studies.[52,53]

9 THE NICANDRA STEROIDS

Another repellent which inhibits the feeding of insect larvae of various species, especially the tobacco hornworm, is found in the leaves of the Peruvian plant *Nicandra physaloides* (L) Gaertn. Although the tobacco hornworm (*Manduca sexta* Joh) normally feeds on Solanaceae plants, it is prepared to die of starvation rather than eat this one member of the group, and the same applies to larvae of other species. Forced feeding the larvae kills them. We discovered seven new steroids in the plant, of which Nic-1 (Fig 16; **35**) is typical of the four unique steroids having aromatic D-rings.[54,55] These have apparently been formed biosynthetically by expansion of the usual five-membered D-ring by inclusion of the adjacent methyl group, followed by aromatisation. Nic 7 (**36**) and the others, whilst still complex, have ring-D in normal 5-membered form.[54,55]

10 THE MAMMEINS

Mamey apples (*Mammea americana* L) are a well-known fruit in the West Indies and their large seeds, about the size of a hen's egg, are ground and used locally as an insect powder. Tests at Rothamsted against mustard beetles (*Phaedon cochlearide* F) showed that the seed extracts were quite insecticidal and a prolonged examination revealed 26 allied coumarins which were inhibitors of oxidative phosphorylation, but not topical insecticides.[56–59] Eventually, however, we isolated two compounds (**37** and **38** of Fig 17) which, whilst being similar in structure to the rest, were also topically insecticidal.[60] The major difference in type was a side-chain acetoxyl in the 1′-position in those which were active insecticides For confirmation of this view I searched for any other natural coumarin of a similar type having such side-chain acetoxylation and found Surangin-B (**39**) in the literature; it had not been tested as an insecticide. We obtained a sample and tests showed it to be if anything more insecticidal to house flies and mosquito larvae than our Mammea compounds.[60] Side-chain acetoxylation thus seems important for the expression of topical insecticidal activity in this series. We have made extensive synthetic studies in the Mammea coumarin area, including mammeas E/BA, E/BB and surangin B.[61–64]

11 DIHYDROAGAROFURAN ESTER ALKALOIDS

A natural product area in which we have published a

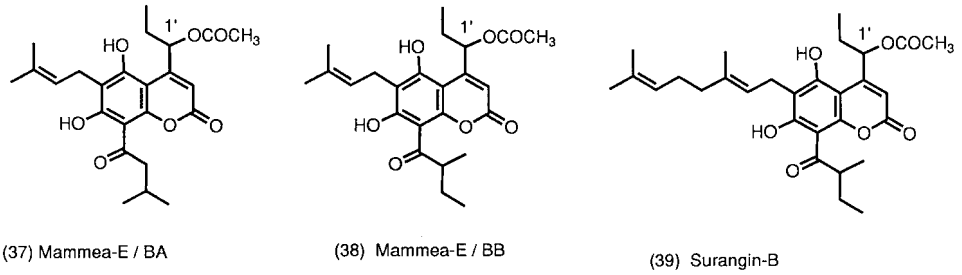


Figure 17. Insecticidal natural coumarins.

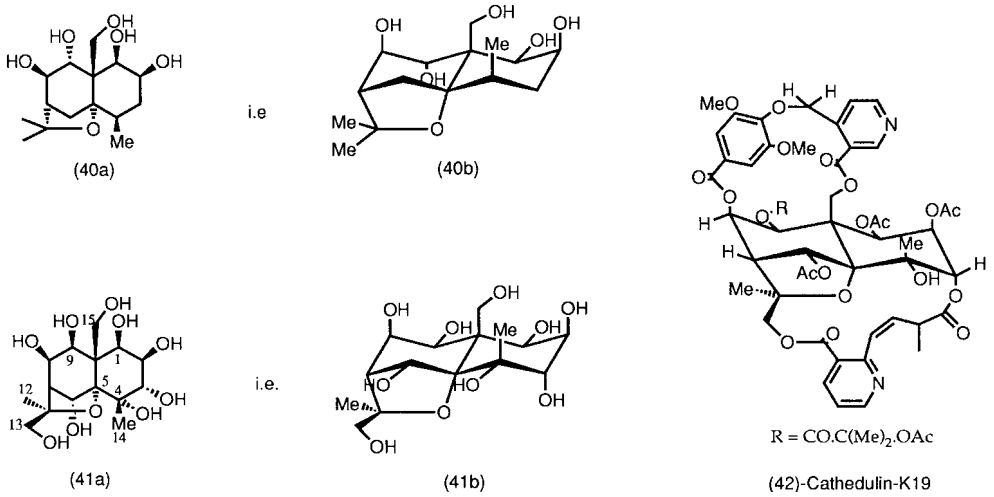


Figure 18. Dihydroagarofuran-type compounds.

good deal is cannabis, but it is not relevant, so far as I am aware, to my theme today. However, it did lead on to our study of another psychotomimetic–the Middle East drug khat (*Catha edulis* Forsk, Celastraceae). Its effect in man is largely due to the presence of simple amphetamine-like compounds such as cathine and especially cathinone.[65] However, our interest was in the group of large alkaloids that we found, of which we were able to isolate and structurally identify 14. They are based on hydroxylated dihydroagarofuran cores (Fig 18; **40, 41**)[66] and their large size is due to heavy esterification, sometimes forming bridges *via* difunctional acids as in the case of Cathedulin K-19 (**42**).[67]

Simpler esters having a dihydroagarofuran core are frequently mentioned in the literature, often with the comment that they are insecticidal. [Spindle, *Euonymus europaeus* L, is a well-known example containing evonine,[68] and there is a classic investigation of the Chinese insecticidal plant *Tripterygium wilfordii* Hook f by Beroza[69]]. We have never had the chance to follow up the insecticidal action of these Celestraceous terpene-based alkaloids, but it would be good to know whether it is the core, or the esters, or a certain combination of both that is responsible for insecticidal effects. More basic biological knowledge of the way in which these and other candidate natural products act on insects is desirable at the present time

THE α- AND β-CEMBRENE DIOLS FROM TOBACCO

The tobacco plant (*Nicotiana tabacum* L) produces a leaf surface gum which is reported to be insecticidal and to have plant growth-inhibiting properties, interfering with the development of axillary shoots.[70–72] Incidentally it also improves tobacco flavour on smoking and, surprisingly, is reported to have anti-tumour-promoting effects. The gum consists largely of α- and β-cembrene diols (duvanes, thunberganes) (Fig 19; **43, 44**) and we have studied their structures and conformation by X-ray analysis.[73] Using tobacco calices rich in trichomes we have shown that [2-^{14}C]geranylgeraniol (**45**) and 3-[^{14}C]cembrene (**46**) are biosynthetic precursors and the cyclopropane casbene (**47**) may precede cembrene.[74] Our synthesis of natural (1*S*, 3*R*)-casbene links back to the chiral chrysanthemic acids as starting materials.[75]

I would now like to turn from our interests in insecticides to our interactions with that other agricultural scourge – fungi.

13 SYNTHESIS OF HOST-SPECIFIC AK AND AS TOXINS

The general toxicity of certain microbiological metabolic products towards plants is of course, well recognised, but host-specific (or better, host-selective)

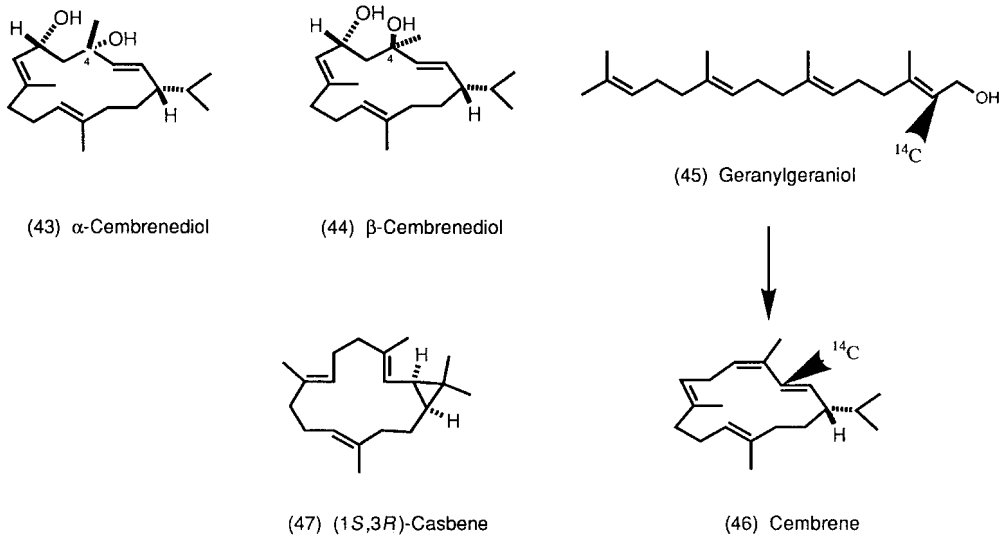


Figure 19. Terpenes associated with tobacco.

toxins (H-S toxins) are highly selective to a plant at the cultivar level and the compounds formed by such microbes are the actual initiators of the plant disease.[76] Benign fungi can become virulent unexpectedly by acquiring the genetic ability to biosynthesise H-S toxin. Such H-S toxins are pathogenic only to their particular hosts, being essentially non-toxic to non-hosts which may yet be closely related cultivars. Spores of the pathogen release a chemically identifiable H-S toxin on germination and it is possible to turn a morphologically identical, but benign, microbial strain into a virulent pathogen merely by providing it with some external H-S toxin.

The activities of H-S toxins in agriculture can be devastating. The introduction in 1927 of the South American oat (*Avena sativa* L) cultivar Victoria into plant breeding led, by the early 1940s, to new cultivars containing genetic resistance to most races of crown rust disease (*Puccinia coronata* Cda) and smut (*Ustilago avenae* Rostr). By the mid 1940s some 80% of the US oat acreage was planted with such cultivars. During 1944 a new oat disease, 'Victoria blight' appeared. It was caused by *Helminthosporium victoriae* Meehan and Murphy and was confined to cultivars containing the Victoria gene; within three to four years the disease was so widespread and devastating that all such cultivars were abandoned. There are a number of similar examples of the devastation caused by H-S toxins.[76]

The H-S toxins we have been particularly interested to synthesise have been those associated with the Japanese pear and strawberries. The fungus causing the disease in pears, *Alternaria alternata* (Fr) Keissl (Japanese pear pathotype), affects Nijisseiki varieties but not other cultivars. Nakashima and his colleagues studied the H-S toxin and characterised two toxic components, AK-toxin I and AK-toxin II, one of which is effective on a susceptible host at 5×10^{-9} M.[77–80] These toxins induce rapid loss of potassium ions from the host cell. Around 1975, a new black-spot disease of strawberry occurred which was also attributed to a pathogenic strain of *A alternata* (strawberry pathotype). The toxins involved are referred to as AF toxins.[80,81]

Our synthetic work has concerned three of these HS toxin types (Fig 20; **48–50**). We worked to a retrosynthetic plan by which the three geometrically isomeric triene alcohol components (**51–53**) were

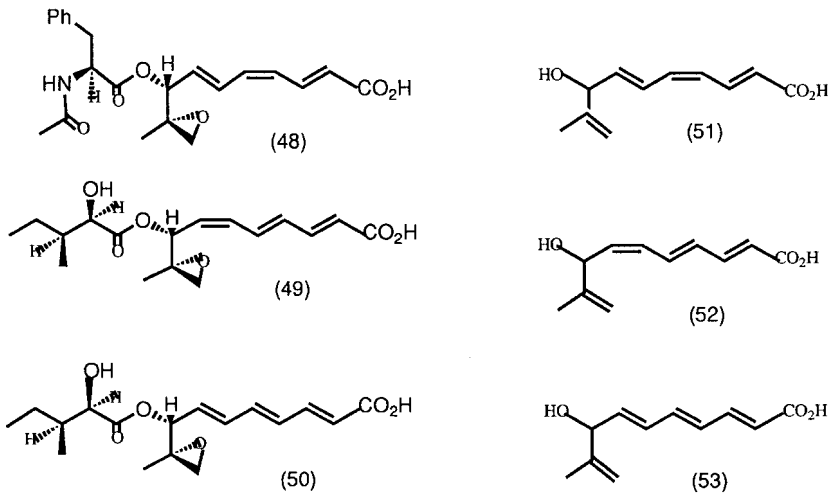


Figure 20. H-S toxins and synthetic intermediates.

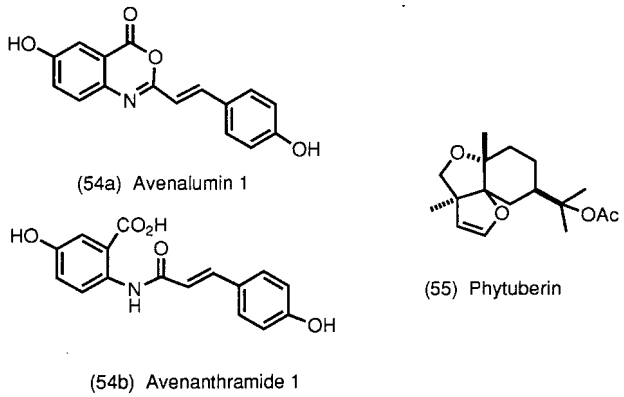


Figure 21. Phytoalexins.

made first, and then epoxidised using chiral epoxidation as introduced by Sharpless.[82] The products were finally esterified with the the appropriate acid. For the synthesis of the trienes we used tin rather than zirconium coupling as it gives better yields and the hydroxyl does not require protection. For a detailed discussion of the actual syntheses the original paper[82] should be consulted. The toxins were all made in chiral form.

14 POTATO AND OAT LEAF PHYTOALEXINS

Just as the fungus has aggressive chemicals, plants have defensive chemicals, and we have done some work using Kornblum nitro-synthesis on the potato phytoalexin (Fig 21; **55**).[83,84] The true phytoalexin of oat leaves, active against crown rust is not the benzoxazinone avenalumin I (**54a**), as had been claimed, but its hydrated form (**54b**).[85]

15 THE AVENACINS: OAT ROOT DEFENCES AGAINST 'TAKE-ALL'

Our main study in the oat area has concerned the chemical defences of oat roots against the fungus *Gaeumannomyces graminis* Arx & Oliv, the causative fungus of 'Take-all' disease.[86] 'Take-all' is a most destructive and widespread disease, attacking the stem bases of susceptible cereals–wheat, barley and rye–and is difficult to control by synthetic fungicides. Oats, however, are not attacked by *G graminis* (var *tritici*) (Ggt), the strain which usually attacks wheat, and infected wheat-land may be cleansed of infection by planting with oats for a year or two. Earlier investigators showed that a fluorescent defensive material was present in oat roots, especially the root tips, but the chemical nature of this material, named avenacin, remained ill-defined.

Our investigation[87] involved hydroponically grown oats, the roots from which were freeze-dried, ground, and extracted with aqueous methanol. Root tips contained 8 μg avenacins per tip. Reversed-phase HPLC led ultimately to the isolation of four pure avenacins, A-1 and B-1 showing an intense blue fluorescence in methanol, whilst A-2 and B-2 were non-fluorescent. All four compounds contained trisaccharide units of the same type formed from 1 mol of arabinose and 2 mol of glucose (Fig 22). The aglycones remaining after acid hydrolysis of the carbohydrate

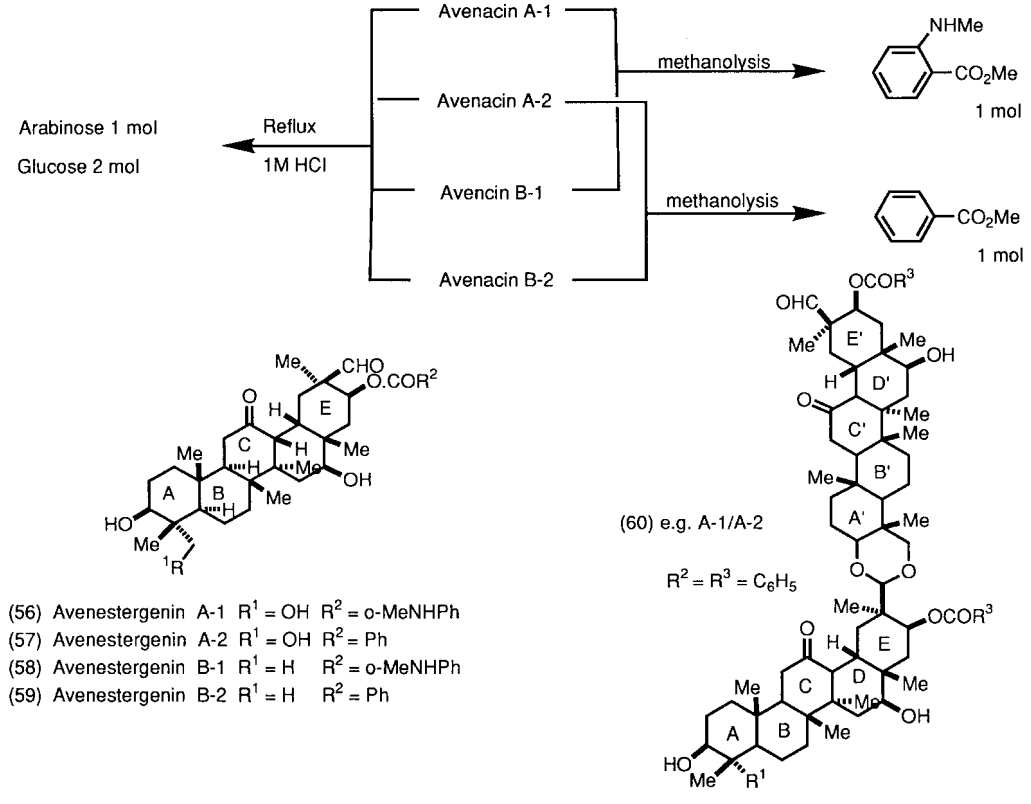
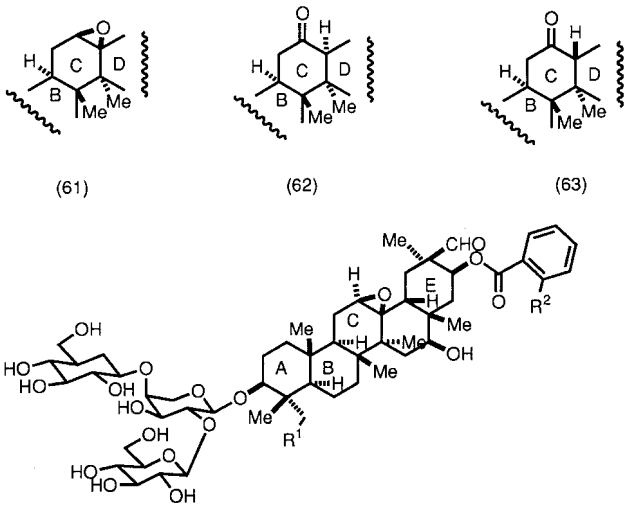


Figure 22. Hydrolysis and methanolysis of avenacins from oat roots.



(61) (62) (63)

(64) Avenacin A-1 R^1 = OH R^2 = NHMe
(65) Avenacin A-2 R^1 = OH R^2 = H
(66) Avenacin B-1 R^1 = H R^2 = NHMe
(67) Avenacin B-2 R^1 = H R^2 = H

Figure 23. The epoxy-ketone rearrangement and the structure of the avenacins.

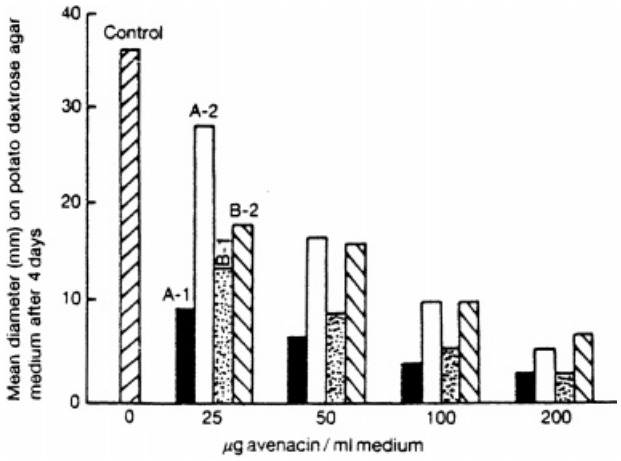


Figure 24. Toxicities of the avenacins towards 'Take-all' fungus (*Gaeumannomyces graminis*) in culture.

were the four avenestergenins (Fig 22; **56–59**) having a 12-carbonyl function, two containing *N*-methylanthranilic acid (hence the fluorescence), and two benzoic acid. Structural work, mainly NMR studies of the aglycones, were later confirmed by X-ray single crystal analysis, and led to complete structures for the four avenestergenins.[87] During the work, blockage of HPLC columns was noticed and it was ascertained that this was due to formation of dimers such as **60**, which could be isolated.[87] However, analysis of spectroscopic data showed that the avenestergenins are apparent, rather than true, aglycones.

During acid hydrolysis a 12,13-epoxide-12-ketone rearrangement, with epimerisation at C-13, (Fig 23; **61** → **62** → **63**), has occurred.[88] The true structures of the four avenacins are thus as shown in Fig 23 (**64–67**) The carbohydrate section was structurally elucidated using mass-spectrometry, [^{1}H]NMR, [^{13}C]NMR and chemical methylation techniques.[88]

The four avenacins strongly inhibit the growth in culture of the wheat 'Take-all' fungus (Ggt), with the *N*-methylanthranilate-containing A-1 and B-1 being more active than A-2 and B-2 (Fig 24).[89] However there is a virulent strain of *G graminis* (var *avenae*) (Gga) which is capable of attacking oats as well as other cereals. We have shown that one of the reasons for the potency of this variant is its ability to hydrolyse first one, and then the second, glucose residue attached to the arabinose.[90] This is clearly a detoxification process, for our tests show that the *mono*- and *bis*-deglucosyl avenacins are considerably less inhibitory to the growth of Gga. It is thought that the avenacins complex with sterols in the fungal hyphae membrane causing aggregation and cytoplasmic leakage through holes. Avenacins are among the most haemolytic saponins known. Wheat losses from 'Take-all' amount to £60 million in the UK alone each year, and the disease is prevalent throughout the world. Ann Osbourn of the Sainsbury Laboratory in Norwich is looking at the genetics of the problem.[91] She has shown that alteration of the genes of the oat plant so that it no longer produces avenacins makes oats susceptible to 'Take-all.' It is hoped that it may be possible to introduce the avenacin-forming genetic equipment into wheat as a protection against 'Take-All' fungus

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